REMARKS/ARGUMENTS

Claims 23, 24, 26 and 28-37 have been examined. By this submission, claims 23, 24, 29 and 31 have been amended. Claims 23 and 31 have been amended to recite the invention with greater particularity. In particular, claim 23 has been amended to recite a composition comprising an isolated cell population having human dendritic cells, wherein said cell population has been cultured in the presence of granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin 4 (IL-4) and exposed *in vitro* to a soluble prostate antigen, the cell population having an increased ability to activate T cells specific to the prostate antigen as compared to an isolated cell population comprising the same number of cells cultured in the presence of granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin 4 (IL-4) that has not been exposed *in vitro* to the prostate antigen. Claim 31 has been amended to conform the language of the claim for consistency with claim 23 upon which it depends. No new matter has been added by the present amendments. Applicants respectfully request reconsideration of the claims currently pending in the application in light of the amendments above and the below remarks.

Objection:

The Examiner has objected to claims 23 and 31 as confusing. In particular, the Examiner has questioned the phrase the "same number of cells that has not been exposed *in vitro* to the prostate antigen" and whether these control cells were cultured in medium containing GM-CSF and IL-4 similar to the cell population having an increased number of human dendritic cells, competent and able to activate T cells specific to the prostate antigen. Applicants in an effort to further expedite prosecution of the claims in the present application have amended claims 23 and 31 to recite that the control cell population has been cultured in the presence of GM-CSF and IL-4 in the same manner and the isolated cell population having dendritic cells that has been exposed to prostate antigen. Applicants believe this amendment to claims 23 and 31 should obviate any confusion about the treatment of the control cell population.

Rejections Under 35 U.S.C. § 112:

Claims 23, 24, 26 and 28-37 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Examiner has summarized claims 23, 24, 26 and 28-37 as being drawn to a composition comprising an isolated cell population exposed *in vitro* to a soluble prostate antigen, the cell population has an increased number of human dendritic cells, competent and able to active T cells specific to the prostate antigen, of at least 20 fold more, as compared to an isolated cell population comprising the " same number of cells that has not been exposed *in vitro* to the prostate antigen." Further, the Examiner does not believe that the specification discloses the limitation of comparing to the "same number of cell" wherein "said cells have not been exposed *in vitro* to the prostate antigen", or a limitation drawn to "at least " 20 fold more."

Although Applicants believe the phrase "having the same number of cells that has not been exposed *in vitro* to the prostate antigen" is taught by the specification, but in order to further expedite prosecution of the present claims, claim 23 and 31 have been amended to recite a composition comprising an isolated cell population having human dendritic cells, wherein said cell population has been cultured in the presence of granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin 4 (IL-4) and exposed *in vitro* to a soluble prostate antigen, the cell population having an increased ability to activate T cells specific to the prostate antigen as compared to an isolated cell population comprising the same number of cells that has not been exposed *in vitro* to the prostate antigen. The cell population that has been cultured in the presence of GM-CSF and IL-4 and also exposed *in vitro* to prostate antigen are shown in Figure 3 to induce a 2 to 3 fold greater antigen specific proliferative T cell response that the control cells that were cultured in the presence of GM-CSF and IL-4 and were not exposed *in vitro* to the prostate antigen. Support can be found for example in the particular embodiment provided in Section 6.3 beginning at page 25 of the specification. The results of these experiments are depicted in Figure 3 wherein 2 of the 4 patients demonstrated significant increases (2 to 3 fold) in T cell proliferation when both dendritic cells and tumor cell lysate were included in the T cell cultures. Neither tumor cell lysate alone or the same number of isolated dendritic cells alone

were sufficient to process and present antigen to the T cells as evidenced by the lack of induction of T cell proliferation by these compositions. These data demonstrate and establish that a cell population having DCs isolated from a patient, cultured in the presence of GM-CSF and IL-4, and exposed to prostate antigen *in vitro* process and present antigen to T cells inducing an antigen specific T cell proliferation response. Whereas, the control cell population that was cultured in the presence of GM-CSF and IL-4 and not exposed to prostate antigen *in vitro* either were unable to process and present antigen to T cells as evidenced by reduced T cell proliferation, or the reduction in T cell proliferation resulted from fewer dendritic cells being present in the cell population competent and active to present antigen.

Further, in Section 7.1, beginning at page 27, dendritic cells were isolated from PBMCs and grown in the presence of GM-CSF and IL-4 for 7 days prior to being cryopreserved. The cryopreserved DCs were stored for a period of time, thawed, and resuspended in medium. Previously frozen T cells isolated from PBMCs obtained from the same cancer patient as the dendritic cells, were either combined with the same number of DCs alone or in combination with purified PSMA. Subsequent to six days of co-culture ^{3}H-thymidine was added for 18 hrs to measure the rate of T cell proliferation. Figure 6 demonstrates that a highly significant increase in thymidine incorporation was observed when both previously frozen DCs and prostate antigen were included in the T cell cultures which substantiates the processing and presentation of prostate antigen by the DCs. The effect was significantly greater than that observed with antigen alone or with the same number of DCs alone but no exogenous prostate antigen.

Applicants believe that the pending claims as amended obviate any rejection set forth by the Examiner as set forth above. Therefore, the Examiner is respectfully requested to reconsider and withdraw the rejection of claims 23, 24, 26 and 28-37 under 35 U.S.C. 112, first paragraph.

Claims 23, 24, 26 and 28-37 also stand rejected under 35 U.S.C. § 112, first paragraph, because the Examiner believes that the specification is enabling for a composition comprising an isolated cell population cultured in the presence of GM-CSF and IL-4 and

exposed *in vitro* to a soluble prostate antigen, the cell population having an increased number of human dendritic cells, competent and able to activate T cells specific to the prostate antigen. The Examiner does not believe that the specification enables any person skilled in the art to which it pertains, or with which it is most nearly connected to make and use the invention commensurate in scope with these claims. In particular, the Examiner does not believe that one can extrapolate the teaching in the specification to the claims, because if the control has the same number of cells that have been cultured in the presence of GM-CSF and IL-4, one would expect that culturing in the presence of GM-CSF and IL-4 would produce in the control the same number of dendritic cells, competent and able to activate T cells specific to the prostate antigen. Further, the Examiner has asserted that one would not expect that exposure of the competent dendritic cells to a prostate antigen would increase the number of the competent dendritic cells, because it is well known in the art that exposure of competent dendritic cells to an antigen only would active the dendritic cells to present the antigen. The Examiner has cited Cohen for this proposition.

Although Applicants do not believe that the claims as previously presented were not enabled by the specification as filed, claims 23 and 31 have been amended to recite the present invention with greater particularity in order to further expedite prosecution of certain aspects of the present invention. As above, claim 23 has been amended to recite a composition comprising an isolated cell population having human dendritic cells, wherein said cell population has been cultured in the presence of granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin 4 (IL-4) and exposed *in vitro* to a soluble prostate antigen, the cell population having an increased ability to activate T cells specific to the prostate antigen as compared to an isolated cell population comprising the same number of cells that has not been exposed *in vitro* to the prostate antigen. Claim 31 has been amended for consistency with claim 23. The amendments to claim 23 clearly set forth that the isolated cells have been cultured in the presence of GM-CSF and IL-4 to increase the number of dendritic cells competent and able to active T cells. The first cell population is contacted with a prostate antigen while the control cell population is not. T cells are activated in an antigen specific manner only by those dendritic cells that have been exposed to prostate antigen and not by the control cells. Applicants do not

believe that Cohen is a proper reference for teaching in the art that exposure of competent dendritic cells to antigen only would activate the competent dendritic cells in an isolated cell population in that the monocytes induced to convert to a dendritic cell-like phenotype are not competent dendritic cells that can take up and process antigen as discussed further below.

Rejections Under 35 U.S.C. § 102:

Claims 23, 24, and 31-36 remain rejected under 35 U.S.C. § 102(e), the Examiner believing the claims to be anticipated by Cohen *et al.*, as evidenced by Sallusto *et al.*, Koski *et al.*, and Czerniecki *et al.* for reasons of record in paper No. 30. In particular, the Examiner believes that Sallusto *et al.* teach that dendritic cells exist in two stages of maturation; immature dendritic cells that are capable of antigen capture/ processing and immunostimulation and mature dendritic cells that lose antigen capturing capacity. Further, the Examiner summarizes the teachings of Czerniecki *et al.* as teaching that human peripheral blood contains a small population of immature dendritic cells distinguished from circulating monocytes by their low expression of CD14. Czerniecki *et al.* is further summarized as teaching that fractions 150-190 of elutriation has an immature dendritic cell staining pattern. Also, the Examiner notes that she believes that Cohen *et al.* teaches that pooled fractions 150, 160, 170, 180 and 190 from patients or donor leukapheresis are incubated for 40 hrs in the presence of autologous tumor cell lysates for both cells untreated and treated with calcium ionophore.

It is the opinion of the Examiner given the teachings of the references as she has interpreted them that regardless of whether the mechanism by which monocytes are converted to the activated dendritic cell-like phenotype is known or unknown a large percentage of cells in the pooled fractions as taught by Cohen *et al.* would inherently comprise immature dendritic cells, as allegedly evidenced by Czerniecki *et al.*, and that this would meet the limitation of the claims. Further, the Examiner has asserted that Cohen also teaches that certain specific combinations of cytokines have been used successfully to amplify or partially substitute for the activation/conversion achieved with calcium ionophore. Thus it is the conclusion of the Examiner that the monocytes treated with a combination of cytokines, which include GM-CSF,

IL-2, IL-4 and IL-12 would also produce immature DCs that maintain the antigen capturing and processing capacity, as the Examiner alleges is evidenced by Sallusto *et al.*

Cohen as previously argued by Applicants teaches a method comprising treating monocytes with a calcium ionophore for the "conversion of the large monocyte population to an activated DC-like phenotype so that they also can participate in effective antigen processing and presentation." Cohen, column 10, lines 30-32. It is further taught by Cohen *et al.* that it is the cells converted to an "activated DC-like phenotype" that are contacted with an antigen and predicted to process and present antigen. Applicants have previously provided extrinsic evidence that these cells having an "activated DC-like phenotype" can not process and present antigen. Koski *et al.* and Zhou *et al.* were cited by Applicants to provide evidence that the cells of Cohen were phenotypically the same as mature activated dendritic cells and therefore would not and could not process and present antigen. The Examiner has now cited Czerniecki *et al.* as teaching that the elutriation method of Cohen produces a large percentage of immature dendritic cells that calcium ionophore treatment converts to dendritic cells. Applicants respectfully wish to point out that contrary to the position of the Examiner, Czerniecki *et al.* do not teach that up to 28% of the combined elutriation product comprises immature dendritic cells. The value cited by the Examiner refers to the percentage of cells displaying the immature dendritic cell staining pattern in certain particular elutriation fractions collected subsequent to the 140 cc/min fraction. Cells that display the immature dendritic cell staining pattern only make up 7 to 10% of the combined elutriation product, while cells displaying the monocyte staining pattern make up 88 to 90%.

Further, Cohen *et al.* do not teach a method of treating cancer by contacting the dendritic cell population provided by the disclosed elutriation method, but instead, as described above, teach a method for converting monocytes to a dendritic cell-like phenotype. There are no teachings in Cohen as to conversion of any other cell type, such as immature dendritic cells. Although Cohen *et al.* contact the elutriation product with tumor lysate prior to contact with calcium ionophore, there is no teaching as to whether these cells process and present antigen. The cells are merely tested for certain phenotypic cell surface markers characteristic of

"activated dendritic cells" or monocytes, see column 11, lines 44-47 and lines 55-58. The conclusion of the experiment states that it demonstrated that calcium ionophore treatment increase the yield of activated dendritic cells by many times over untreated cell fractions, see column 11, lines 65-67. Cohen proceeds to teach in the method for treating cancer that subsequent to "activation by 500 ng calcium ionophore A23187. The pooled fraction are then incubated for 40 hours with a prostate tumor cell lysate from prostate cancer cells previously obtained from a biopsy of the patient." Activation as set forth above is the conversion of monocytes to the dendritic cell-like phenotype which is unable to process and present antigen.

Applicants do not believe that the later discovery of the presence of immature dendritic cells by Czerniecki *et al.* adds anything which might fill any gap in Cohen *et al.* Even if immature dendritic cells are present and convert as taught for monocytes, the converted immature dendritic cells would not take up and process antigen just as would be the case for the converted monocytes. Further, as immature dendritic cells make up such a small percentage of the total cell population in the elutriation product early addition of antigen for processing by the immature dendritic cells prior to or during conversion, assuming for *argumento* that such processing does occur, would be equivalent to a control culture with no increase in the ability to induce the activation of antigen specific T cells. Applicants assert that Czerniecki *et al.* merely note that immature dendritic cells are present in the elutriation product taught by Cohen *et al.*. Further, Cohen *et al.* do not teach or suggest the fate of any cell other than the monocytes. Therefore, the skilled artisan at the time of the present invention, viewing the process as taught by Cohen *et al.* would have added antigen to the "activated dendritic-like cells," and as above, these "activated dendritic-like cells" are mature dendritic cells that would be unable to process a soluble prostate antigen as previously argued by Applicants.

The Examiner has also again referred to the portion of Cohen that discusses the use of certain specific combinations of cytokines that have been used in culturing dendritic cells. Applicants again wish to point out the Cohen has compared various cytokines to be inferior to the use of calcium ionophore in "converting monocytes to an activated dendritic cell-like phenotype" and that certain undefined combinations have been used to amplify (or partially

substitute) for the activation/conversion achieved with calcium ionophore. This teaching does not anticipate the present invention as no specific combination is cited. Further, and as discussed above the term "activation/conversion" as used by Cohen refers to mature dendritic cell state at the conclusion of their process and that these activated cells can not take up and process antigen.

Therefore, in view of the remarks above, Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 23, 24, and 31-36 under 35 U.S.C. § 102, as anticipated by Cohen *et al.* as evidenced by Sallusto *et al.*, Koski *et al.*, and Czerniecki *et al.*

Rejections Under 35 U.S.C. § 103:

Claims 23, 24, and 31-36 stand rejected under 35 U.S.C. § 103 as being obvious over Cohen *et al.*, in view of Sallusto *et al.* and Inaba *et al.* (*J. Exp. Med.* 166:182-194 (1987)). The Examiner has summarized the teachings of Cohen *et al.* as the isolation of dendritic cells from peripheral blood from a human normal individual and from a cancer patient by leukapheresis and centrifugation. Further, Cohen *et al.* is stated to teach that pooled fractions 150, 160, 170, 180 and 190 from patients or donor leukapheresis were incubated for 40 hours in the presence of autologous tumor cell lysate, for both cells untreated and treated with calcium ionophore (columns). Also, Cohen *et al.* is alleged to teach that certain specific combinations of cytokines have been used successfully to partially substitute for the activation/conversion of isolated cells to dendritic cells as achieved with calcium ionophore. The cytokines included rhGM-CSF, rhIL-2, reIL-4 and rhIL-12, and that each cytokine when used alone was inadequate. The Examiner further states that Cohen *et al.* teach that the enriched dendritic cells are incubated with a prostate tumor lysate which activates the dendritic cells to present prostate tumor antigens and that Cohen *et al.* also teach that the dendritic cells after incubation with the tumor lysate can be reintroduced to a patient to reduce the size of a tumor. Cohen *et al.* is not believed by the Examiner to teach that the number of dendritic cells are at least 20 fold more as compared to an isolated cell population comprising the same number of cells that has not been exposed *in vitro*

to the prostate antigen, that the dendritic cells are immature dendritic cells, or that the activated T cells are $CD4^+$ or $CD8^+$.

Sallusto *et al.* is described by the Examiner as teaching that dendritic cells exist in two stages of maturation including immature dendritic cells that can take up and process antigen and mature dendritic cells lose antigen-capturing capacity. Further, the Examiner alleges that Sallusto *et al.* teach that the exposure to GM-CSF plus IL-4 converts blood mononuclear cells to immature dendritic cells that could efficiently present soluble antigen, such as tetanus toxoid to specific T cell clones. Sallusto *et al.* is also believed to teach that cells grown with a combination of GM-CSF and TNFα are inferior to those obtained with GM-CSF and IL-4.

Inaba *et al.* is cited by the Examiner for teaching that dendritic cells can activate $CD4^+$ and $CD8^+$ T cells.

The Examiner asserts that it would have been *prima facie* obvious to a person of skill in the art at the time the invention was made to obtain dendritic cells, comprising exposing blood mononuclear cells with a combination of cytokines comprising rhGM-CSF, rhIL-2, reIL-4 and rhIL-12, or with GM-CSF and IL-4 as taught by Cohen *et al.* and Sallusto *et al.* and that one of skill in the art would have expected that the dendritic cells are immature dendritic cells that can effectively present soluble antigen, such as prostate cancer antigen, and are able to activate specific T cells, because Sallusto *et al.* allegedly teaches that by culturing in GM-CSF and IL-4 dendritic cell lines have been established that maintain the antigen capturing and processing capacity characteristics of immature dendritic cells *in vivo*, and because Cohen *et al.* teach exposure of dendritic cells to prostate tumor cell lysate to activate the dendritic cells to present prostate tumor antigens. Further, the Examiner believes that one of skill in the art would have expected that the yield of dendritic cells taught by Cohen and Sallusto would be at least 20 fold more as compared to an isolated cell population comprising the same number of cells that have not been exposed *in vitro* to the prostate antigen, because the immature dendritic cells taught by Sallusto and Cohen seem to be produced by the "same process as disclosed in the specification of the instant invention." It is also the belief of the Examiner that one of ordinary skill in the art

would have expected the dendritic cells as taught by Cohen would activate $CD4^+$ and/or $CD8^+$ T cells because the activation of these cells in taught by Inaba *et al.*

Applicants must respectfully disagree with the summary of the references and with the conclusions as to what would be the expectations of the skilled artisan at the time of the present invention. In particular, Cohen as set forth above mention the prior use of certain cytokines in the successful amplification (or partial substitution) for activation/conversion achieved with calcium ionophore. This disclosure does not teach the use of any combination of cytokines as set forth by the Examiner. Further, as set forth above, Cohen teaches that activation/conversion of monocytes to a dendritic cell-like phenotype. To date the Examiner has yet to set forth where in Cohen or in any other reference this cell population is taught to take up and process a soluble antigen. The Examiner has stated that immature dendritic cells are present in large amount numbers in the elutriation fractions as taught by Cohen (28%), but the immature dendritic cells actually only make up only 7 to 10% of the pooled elutriation fractions. The Examiner has also speculated that the monocytes might process antigen during conversion, but has provided no support for this speculation. Cohen does combine the elutriation fractions with tumor cell lysate, but does not provide any evidence that the lysate provides any antigen that is processed and presented. The only method taught by Cohen is the addition of the tumor cell lysate after "uniform activation" and dendritic cell phenotype conversion of the cell population. As set forth above and in prior responses the activated/converted cells of Cohen can not take up and process antigen. Therefore, there is nothing to suggest the combination of Cohen with either Sallusto or Inaba. Even should the teachings be combined at most it might suggest the addition of an antigen such as tetanus toxoid subsequent to activation/conversion of the monocytes and as the cells would be unable to process the antigen to activate any T cell.

Claim 26 stands rejected under 35 U.S.C. § 103 as being obvious over Cohen *et al.* in view of Sallusto as applied to claims 23 and 24, and further in view of Lutz *et al.* for the reasons of record. In particular, the Examiner believes it would have been *prima facie* obvious to a person of skill in the art at the time the invention was made to immortalize the dendritic cells as taught by Cohen *et al.* and Sallusto *et al.* using the methods for the immortalization of cells

taught by Lutz *et al.* As above, Applicants have demonstrated that Cohen *et al.* nor Sallusto *et al.* neither teach not suggest the dendritic cells of the present invention. Therefore, Lutz *et al.* adds nothing to render obvious the immortalized dendritic cells of the present invention.

Claim 28 and 29 stand rejected under 35 U.S.C. § 103 as being obvious over Cohen *et al.* in view of Sallusto *et al.* as applied to claims 23 and 24, and further in view of Taylor *et al.* for the reasons of record. In particular, the Examiner believes that it would have been *prima facie* obvious to a person of skill in the art at the time the invention was made to cryopreserved the dendritic cells taught by Cohen *et al.* and Sallusto *et al.* using the cryopreservation methods taught by Taylor *et al.* and that one of skill in the art would have been motivated to do so, to preserve the previously isolated dendritic cells. As above, Applicants have demonstrated that neither Cohen *et al.* nor Sallusto *et al.* disclose or suggest the dendritic cells of the present invention. Therefore, Taylor *et al.* adds nothing to render obvious the preserved cells of the present invention.

Claim 30 stands rejected under 35 U.S.C. § 103 as being obvious over Cohen *et al.* in view of Sallusto *et al.* as applied to claims 23 and 24, and further in view of Taylor *et al.*, as applied to claims 28 and 29, and Lutz *et al.* for the reasons of record. In particular, the Examiner believes it would have been *prima facie* obvious to a person of skill in the art at the time the invention was made to immortalize the cryopreserved dendritic cells taught by Cohen *et al.,* Sallusto *et al.*, and Taylor *et al.* using the immortalization methods of Lutz *et al.*, because immortalizing dendritic cells would overcome the problem of being unable to maintain dendritic cells *in vitro* for long periods of time. As above, Applicants have shown that neither Cohen *et al.* nor Sallusto *et al.* do not disclose or suggest the dendritic cells of the present invention. Therefore, there is no motivation for the skilled artisan to combine the references as suggested by the Examiner.

In view of the amendments and remarks above, Applicants respectfully request the Examiner to reconsider and withdraw the various rejections of claims 23, 24, and 31-36 under 35 U.S.C. § 103 as being obvious over Cohen *et al.*, in view of Sallusto *et al.* and Inaba *et*

al. Further, as Applicants believe that the claims have been demonstrated to be non-obvious over the primary references, it is further requested that the various rejections of the claims under 35 U.S.C. § 103 in view of each of the cited combinations of references also be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

20 February 2004

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